THE DEMONSTRATION OF IMMUNE OPSONINS FOR THE PLEOMORPHIC STREPTOCOCCUS IN EXPERIMENTAL POLIOMYELITIS IN MONKEYS

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Rosenow, Towne and Wheeler,[1] Mathers,[2] Nuzum and Herzog,[3] and Kolmer, Brown and Freese,[4] have described a micrococcus isolated quite constantly from brain and cord in epidemic poliomyelitis, and Rosenow and Towne[5] have isolated a similar organism from paralyzed monkeys following the injection of poliomyelitic virus. Mathers and Tunnicliff[6] found an increase in opsonin apparently specific for this micrococcus in the serum of patients during the attack of poliomyelitis, and Mathers and Howell[7] found a specific increase in opsonin in the serum of rabbits immunized with different strains of the pleomorphic streptococcus. Kolmer and Freese[8] using polyvalent antigens of this streptococcus obtained complement fixation with the serum of a small percentage of persons with poliomyelitis. Solis-Cohen and Heist[9] found "that the serums of a large percentage of patients with poliomyelitis give high opsonic indexes with this streptococcus but not with streptococci from nonpoliomyelitic sources nor with staphylococci, diphtheroids and gram-negative bacilli obtained from poliomyelitic material."

In an extensive study of the question of antibody production in poliomyelitis, Rosenow and Gray[10] found an increase in the specific agglutinating power toward this organism in the serum of patients with poliomyelitis, in the serum of monkeys with poliomyelitis following inoculation of virus in the usual way, and in the serum of monkeys injected with the "poliococcus." The present study concerns the development of opsonins in the serum of monkeys following the inoculation of poliomyelitis virus.

[1] Jour. Am. Med. Assn., 1916, 67, p. 1202.
[2] Jour. Am. Med. Assn., 1916, 67, p. 1019. Jour. Infect. Dis., 1917, 20, p. 113.
[3] Jour. Am. Med. Assn., p. 1205.
[4] Jour. Exper. Med., 1917, 25, p. 789.
[5] Jour. Med. Research, 1917, 36, p. 175.
[6] Jour. Am. Med. Assn., 1916, 67, p. 1935.
[7] Jour. Infect. Dis., 1917, 21, p. 292.
[8] Jour. Immunol., 1917, 2, p. 327.
[9] Jour. Infect. Dis., 1918, 22, p. 175.
[10] Ibid., p. 345.

Four monkeys were used in the experiment.

Monkey 147, a normal control, was not injected.

Monkey 148, April 21, 1917, was given 0.5 c c of a 5% suspension in salt solution of glycerolated virus intracerebrally. April 28 the animal showed paralysis; it died April 29. The lesions were characteristic and the pleomorphic streptococcus was isolated from brain and cord.

Monkey 149, April 21, 1917, was given 5 c c of sensitized vaccine intravenously; the dose contained streptococci from 75 c c of dextrose broth culture; the organisms had been suspended in immune horse serum for 2 hours at 37 C. and left in the icebox over night; they were then washed in water and suspended in salt solution.

Monkey 150, April 21, 1917, was given 20 c c of normal horse serum intravenously to test whether horse serum alone would produce any increase in opsonin. May 2, four days after the death of Monkey 148, Monkey 150 was given 0.5 c c of a 5% saline solution suspension of glycerolated virus intracerebrally, and in addition an intravenous injection of 12 c c of immune horse serum. The intravenous injection of 12 c c of immune horse serum was repeated May 3, 5, 6, and 7. May 8, the animal showed definite flaccid paralysis, and died May 14. The lesions were characteristic; the results of the cultures were similar to those from Monkey 148.

Blood was collected from these 4 monkeys April 18 and every 2nd day thereafter until April 29 when the paralyzed monkey died. The blood was allowed to clot, placed in the icechest for 24 hours and after the serum had been decanted it was stored in the icebox until June when the counts were made.

TECHNIC

The strains of pleomorphic streptococci used were shown to have retained their specific agglutinating property. They came from cases of epidemic poliomyelitis occurring in New York and Philadelphia, and from monkeys paralyzed by the injection of virus. Eight of the human strains (714, 721, 722, 729, 839, 841, 842, 899) were recovered from the brain and cord and 3 (730, 732, 748) from the tonsils. The 3 monkey strains (M49, M106, M148) were recovered from the brain and cord of monkeys paralyzed with virus or filtrates of virus. The exponents to the right and above the figures designating the strain in Tables 1 and 2 indicate the number of animal passages; the figure following the period designates the culture generation. Three control strains were used, a streptococcus viridans from the tonsil in a case of arthritis, a pneumococcus (622), and a feebly hemolytic streptococcus (257).

Most of these strains had been kept in the laboratory in deep stabs of ascites fluid, plain tissue agar and were transferred to broth 24 hours before the counts were made. Any broth cultures in which the growth was not uniformly diffuse were discarded.

The test tube method was used in making the opsonin determinations. The tubes, containing 0.05 c c each of leukocyte suspension,

serum, and culture, were incubated at 37 C. for 15 minutes, after which the smears were made immediately. The organisms in 50 leukocytes were counted; the figures in the tables, therefore, represent the actual number of organisms taken up by 50 leukocytes. Any cell which contained more than 30 organisms was not included in the count. Almost without exception these crowded leukocytes were found in mixtures containing immune serum.

The bacterial counts made are tabulated in series, 2 series in Table 1, and 3 in Table 2. As the same 24 hour culture, and the same leukocyte suspension were employed throughout, the conditions for each single series were uniform.

TABLE 1

OPSONIC POWER OF THE SERUM OF 4 MONKEYS

Strain	Monkey 147 Normal Control		Monkey 148 Injected with Virus		Monkey 149 Injected with Sensitized Vaccine		Monkey 150 Injected with Normal Horse Serum	
			Before Injection	Eight Days after Injection; One Day after Onset of Paralysis	Before Injection	After Injection	Before Injection	After Injection
	April 18	April 29	April 18	April 29	April 18	April 29	April 18	April 29
899	51	50	39	257	32	65	61	56
M 106.5	38	2	14	120	0	68	0	4
$722^{2}.4$	56	20	20	91	22	119	66	54
730.11	87	59	39	139	19	78	55	54
913 control	17	..	19	12	47	51	10	10
714.3	..	..	2	43	9	57	17	6
$714^{3}.2$	..	..	22	64	20	91	64	32
$714^{4}.2$	..	..	0	20	10	35	4	4
$748^{2}.4$	..	..	30	64	18	30	14	12
622 control	..	..	34	38	32	24	10	11

In Table 1 is given the opsonic power of the serum of 4 monkeys obtained in 2 series of experiments. The serum of the normal control, Monkey 147, and the one injected with normal horse serum, Monkey 150, in no case shows any increased opsonic power. On the other hand, the opsonic power of the serum of Monkey 148 injected with virus, and Monkey 149 injected with sensitized vaccine, shows a well marked increase in phagocytic power 8 days after the injection against all of 7 human strains of the pleomorphic streptococcus (3 before and 4 after from 1-4 animal passages) and one monkey strain, but no increase against the control strains.

TABLE 2

RESULTS SECURED IN 3 ADDITIONAL SERIES OF EXPERIMENTS

Strain	Opsonic Power of Serum													
	Monkey 147 Normal Control		Monkey 148 Injected with Virus					Monkey 149 Injected with Sensitized Vaccine		Monkey 150 Injected with Horse Serum and Virus				
			Before Injection	Two Days after Injection	Four Days after Injection	Six Days after Injection	Eight Days after Injection; One Day after Onset of Paralysis	Before Injection	After Injection	Before Injection	Two Days after Injection of Serum	Six Days after Injection of Serum	Twelve Days after Injection of Serum	Twelve Days after Injection of Virus
	April 23	May 2	April 20	April 23	April 25	April 27	April 29	April 18	May 2	April 18	April 23	April 27	May 2	
M 148.3	18	17	1	..	..	..	62	7	14	..	29	..	19	
721	49	26	32	..	..	..	131	24	41	..	58	..	44	
729	60	40	27	..	..	..	85	22	79	..	43	..	45	
730	40	18	22	..	..	..	85	28	65	..	32	..	27	
839	106	41	..	105	..	..	191	53	154	..	34	..	37	
842	24	4	..	10	..	..	55	...	...	..	12	..	10	
M 49.6	24	..	31	..	..	..	53	40	64	44	..	..	30	
M 148	14	..	10	.	..	..	23	25	36	10	..	..	12	
732.9	24	..	12	..	..	..	51	49	65	18				
257 control	18	.	5	..	..	..	..	32	16	18	..	..	50	
899	..	..	..	..	74	68	257	...	...	..	..	..	68	344
730	..	..	..	..	25	26	60	...	...	..	..	..	7	64
839	..	..	..	..	107	92	249	...	...	..	..	..	81	171
841	..	..	..	..	38	82	127	...	...	..	..	..	63	329
714[3.2]	..	..	..	..	18	..	116	...	...	..	..	..	17	80

In Table 2 are given the results obtained in 3 additional series of experiments. In this series, as in those shown in Table 1, the opsonic power of the serum of the normal control (Monkey 147) and Monkey 150 varied only slightly. The serum of Monkey 148 again shows a marked increased opsonic power after injection, and after paralysis occurred. The increase is slight up to the 6th day after the injection of virus, but decided between the 6th and 8th days. The serum of Monkey 149 shows a consistent increase in opsonic power with all of 9 strains 14 days after the injection of sensitized vaccine. It is of interest to note that this monkey showed a degree of immunity to virus in that the incubation period was prolonged for 1 week and the animal recovered, while the control died promptly after the onset of paralysis.

The serum of Monkey 150 showed no change in opsonic power in numerous tests until 12 days after an intracerebral injection of virus and after repeated injections of immune horse serum. At this time there was a marked rise toward all of 5 strains (Table 2), and coincident with this marked increase in opsonin, the animal showed a degree of immunity to virus, since it lived for 6 days after the onset of paralysis, while the control died of a rapidly progressing paralysis in 24 hours. It has previously been observed that immune horse serum does not always protect completely against the forced experiment of intracerebral inoculation of highly virulent virus. The lack of complete protection in Monkey 150 may, however, be due to the fact that the immune horse serum may have been toxic since the animal received an injection of normal horse serum 12 days previously (Tables 1 and 2).

CONCLUSIONS

There occurs a well marked specific increase in opsonin for the pleomorphic streptococcus in the serum of monkeys during attacks of poliomyelitis following the inoculation of virus. Since this increase in opsonin occurs toward strains derived from human cases as well as from experimental poliomyelitis following the injection of virus, the pleomorphic streptococcus in this disease cannot be regarded as an accidental invader of the nervous system.